Journal of Materials Chemistry B

Accepted Manuscript

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Journal of Materials Chemistry B RSCPublishing

Highlights

Amyloid-Directed Assembly of Nanostructures and Functional Devices for Bionanoelectronics

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2015,
Accepted 00th January 2015

DOI: 10.1039/x0xx00000x

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Amyloids have the potential to serve as useful building blocks for functional materials and bionanoelectronic devices because they possess precisely assembled fibrillar structures, tunable functionalities, excellent mechanical properties, and ultrastability over a wide range of harsh conditions. These attributes endow amyloids with the ability to direct one-dimensional (1D) and two-dimensional (2D) patterning of nanoparticles and to serve as templates for controlled growth of nanowires, nanotubes and hybrid architectures. When coupled with conductive objects or functionalized with particular molecules, peptides or protein domains, amyloids could become active components of biosensors and devices relevant to energy applications. Before the full potential of amyloids in future bionanoelectronics can be realized, however, key engineering breakthroughs will be required.

Introduction

The field of bionanoelectronics is a broad interdisciplinary field that requires knowledge and techniques from chemistry, physics, engineering, biology, and medicine.[1, 2] Two overarching goals of bionanoelectronics are nanostructurally precise fabrication and device integration for technical applications. Although conventional top-down fabrication will continue to play a role, a bottom-up strategy of self-assembly is a promising approach for constructing devices with reduced size features.[3] Biomolecules such as DNAs and proteins are particularly suitable for such applications because they are intrinsically capable of molecular self-assembly and the resulting nanoscale objects can further direct the organization of nanoelectronic building blocks such as nanoparticles (NPs), nanowires, nanotubes or complex nanostructures in a spatially controlled manner.[4-8] However, most naturally occurring molecules (e.g., DNAs, proteins, peptides, and lipids) have notoriously low stabilities over many harsh conditions (e.g. high temperature and low-moisture conditions), thereby more or less limiting their practical applications in bionanoelectronics.

Amyloids, one type of protein structures, are characterized by β-strands that are oriented perpendicularly to the fibril axis and connected through a dense hydrogen-bonding network, leading to supramolecular β-sheets that usually extend continuously over thousands of molecular units.[9] In addition to their well-defined molecular self-assembly, such fibrillar structures have intrinsic advantages for constructing functional materials and molecular devices with excellent mechanical properties,[10] tunable molecular functionality,[11] binding affinities,[12] and ultrastability over high temperature, low moisture, protease, and acidic or basic conditions.[13] These attributes make amyloids more attractive objects for bionanoelectronics, particularly for applications that must function well under high temperatures and other harsh conditions, compared with many naturally occurring molecules typically with low stability. In this mini-review, we summarize and discuss recent advances for amyloid-enabled bionanoelectronic techniques based upon amyloid proteins of diverse origins. Two aspects, amyloid-directed assembly of nanostructures and amyloid-enabled functional devices are highlighted to show the promises of amyloids for future bionanoelectronics.

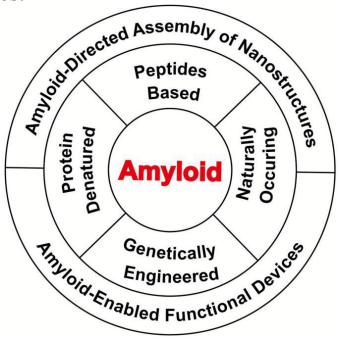


Figure 1. Amyloids as building blocks for bionanoelectronics. Amyloid-directed assembly of nanostructures and amyloid-enabled functional devices could be achieved based on amyloid fibres of different origins, including peptides, denatured proteins, naturally occurring amyloids and genetically engineered amyloid structures.

Amyloid-directed assembly of nanostructures

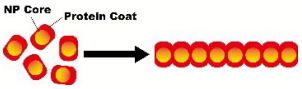
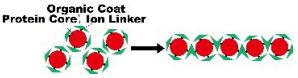
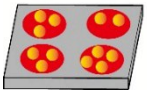
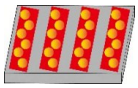
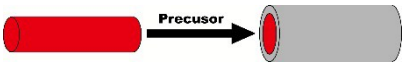
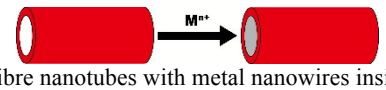
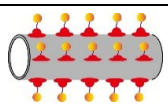
There are two main strategies for applying amyloids to direct nanostructures assembly: (1) amyloid-directed 1D or 2D patterning of nanoparticles; (2) amyloid-templated synthesis of metal, metal-oxide and organic nanowire/nanotube structures—a biomimetic mineralization strategy (Table 1).

In the first strategy, amyloids having specific amino acid residues, functional groups or molecular recognition moieties could be coupled with their 1D fibre feature to order metal nanoparticles (NPs) or quantum dots (QDs) into 1D aligned nanostructures. Specific examples include fibrils made of surfactant-like oligopeptides $(Ala)_{10}$-$(His)_6$ binding to functional gold nanoparticles through His-tags,[14] fibres from denatured hen egg

white lysozyme aligning citrate-capped gold nanoparticles on glass and silicon substrates,[15] Fibronectin nanofibrils covalently attached with CdSe-ZnS core-shell quantum dots,[16] and genetically engineered curli fibres directing the assembly of Au NPs and CdTe/CdS QDs hybrid structures along one single fibre.[17] In other cases, nanoparticles or organic molecules can first be coated or decorated with amyloid monomers and spontaneously self-assembled into aligned nanostructures through amyloid monomer interactions.[18, 19] For example, Lee et al. demonstrated that Au nanoparticles coated with amyloidogenic α-synuclein monomers, like pea-pod-type building blocks, would self-assemble into anisotropic chains in hexane or acidic solution.[19]

Amyloid fibres can also form ordered film structures to organize various nanoparticles into 2D patterned structures on various substrates.[20, 21] For example, a genetically modified protein based on hydrophobin, NCysHFBI, could first form ordered monolayers on pre-patterned Si/SiO_2 surface through hydrophobic interactions.[21] Gold nanoparticles can then bind to the patterned monolayers through cysteine/gold interactions. Large-scale patterning of gold nanoparticles in a controlled manner could thus be achieved.[21] Similarly, organized protein monolayers composed of amphiphilic fibrils could induce the formation of ordered Au nanoparticle chains on mica substrate, facilitated by hydrogen bonding interactions between thymine groups on fibrils and decorated adenine groups on gold surfaces.[20]

Table 1. Amyloid-directed assembly of nanostructures through (1) amyloid-directed 1D or 2D patterning of nanoparticles and (2) amyloid-templated synthesis of nanowires, nanotubes and hybrid nanostructures. Note: The red color represents amyloid proteins in all cases.

Types of amyloids	Functional objects	Features of assembled nanostructures
$(Ala)_{10}$-$(His)_6$,[14] Lysozyme,[15] CsgA-SpyTag[17] Fibronectin[16]	Au NPs[14, 15, 17] CdTe/CdS quantum dots[17] ZnS quantum dots[17] CdSe-ZnS quantum dots[16]	 Aligned 1D structures
α-synuclein[19]	Au NPs[19]	 1D Nanowires assembled from decorated particles
α-synuclein[18]	Fe^{3+}/Phthalocyanine Tetrasulfonate[18]	 1D Nanowires assembled from decorated amyloid proteins
NCysHFBI[21]	Si/SiO_2/Au NPs[21]	 Patterned nanoparticles on substrates
Ac-$(DL)_2$-[D-L$(Thy)_x(Ac)_{1-x}$]-$(DL)_5$-PEG_{70}[20]	Mica/Au NPs[20]	 Aligned 1D structures on substrates
$(VK)_4$-VPPT-$(KV)_4$[22]	Au NPs[22]	 Laminated structures with NPs intercalated
Sup35p,[23] Hen Egg White Lysozyme,[24] Bovine β-lactoglobulin,[25] Insulin.[26, 27] α-synuclein[28, 29]	Au NPs[23] Polyaniline[24] TiO_2[25] Pt[26] PdO[27] ZnS[28] CdS[29] PbS[29]	 1D nanowires formed using fibres as templates
Diphenylalanine[30]	Ag[30]	 Fibre nanotubes with metal nanowires inside
Diphenylalanine[31]	Ag/Au[31]	 Metal-insulator-metal coaxial nanotubes
NCysHFBI[32]	Au/SWNTs[32]	 Carbon nanotubes with NPs aligned

In addition to the 1D and 2D patterns, Lamm et al. demonstrated that a rationally designed amphiphilic peptide could produce periodically spaced, parallel, linear nanoparticle arrays in a layer-by-layer format.[22] Gold nanoparticles were immobilized within such laminated β-sheet fibrils through electrostatic interaction between negatively charged nanoparticles and positively charged lysine residues.

In the second strategy, amyloids were often employed as mineralization templates to deposit metal or semi-conductor compositions thereby forming nanowires, nanotubes and other nanostructures. This strategy was actually inspired by the observations that biomolecules in biominerals can serve as templates or soluble molecules to direct the formation of inorganic compositions into hierarchical structures such as $CaCO_3$ in mollusk shells.[33]

The most common structures based upon this strategy were probably metal, metal-oxide and organic nanowire/nanotube structures. Scheibel et al. reported the first example of applying amyloid fibres as templates to prepare metal nanowires.[23] Au nanowires could form on the surface of a yeast Sup35p protein fibre once metal salts were reduced.[23] Gazit et al. reported that silver ions in the presence of citric acid would be reduced to form nanostructures within diphenylalanine nanotubes.[30] Ag nanowire could thus form following enzymatic degradation of the peptides. Taking this one step further, the same group proposed a method to produce hybrid structures made of metal-insulator-metal, trilayered, coaxial nanotubes.[31] Other interesting examples include single-crystal platinum nanowires and PdO nanowires templated by insulin fibrils,[26, 27] TiO_2 nanowires by β-lactoglobulin fibres,[25] polyaniline nanowires using hen egg white lysozyme as a template,[24] ZnS nanoparticle chains, CdS and PbS semiconducting nanoparticle chains through mature α-synuclein fibrils.[28, 29]

In addition to preparation of nanowires or nanotubes, amyloid fibres can be utilized to solubilize and functionalize carbon-based materials. Kurppa et al. showed that the hydrophobic patch of a NCysHFBI protein could solubilize and bind single-walled carbon nanotubes (SWNTs), while cysteine at N-terminus could form covalent bond with monomaleimido nanogold.[32] As a result, they have successfully synthesized carbon nanotubes/gold nanoparticles hybrid materials, with gold nanoparticles aligning along carbon nanotubes.

Amyloid-enabled functional devices

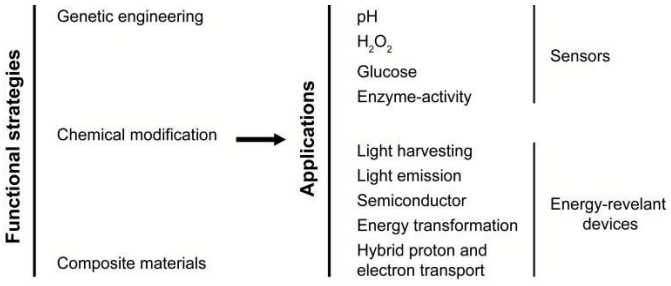


Figure 2. Functional strategies for constructing amyloid-enabled biosensors and amyloid-based energy-relevant devices, and various demonstrated applications associated with these two types of devices.

Amyloids can be integrated into functional devices for both molecular detection and diagnostics and for energy applications, which we shall here refer to as biosensors and energy-relevant devices, respectively (Figure 2). In both, amyloids can be combined with conductive components to form hybrid functional devices. Alternatively, amyloids themselves can serve as active components for functional devices by conjugating or fusing functional molecules/domains through chemical modifications or gene engineering.

In a typical amyloid-enabled sensing device, amyloids are coupled with conductive materials to form hybrid devices. In other cases, amyloids can be integrated with functional organic molecules to achieve a multi-composition device for specific molecular detection. Li et al. showed that hybrid films composed of amyloid fibres and 2D gold nanoplatelets can act as a biosensor to detect pH of solutions.[34] Moreover, Sasso et al. revealed that whey protein amyloid fibres could bind both gold and glucose oxidase through biotinylation and thiolation.[35] Such composite materials could be used for detecting glucose in solution.

Because carbon-based materials such as graphene and carbon nanotubes have high conductivity and excellent mechanical property, researchers have attempted to couple these materials with amyloids for building functional devices.[36, 37] Ultrathin strong hybrid films made of graphene nanosheets and peptide amyloid exhibited excellent conductivity, reversible shape-memory effect and were applied for enzyme-activity sensing.[36] Similarly, a hybrid device composed of peptide-based amyloids, Ag nanoparticles, and graphene nanosheets could serve as a sensor to detect H_2O_2.[37] This device showed better detection sensitivity than Ag nanoparticles-only device.

Beyond sensors, amyloids have been shown to have potential for constructing energy-relevant materials or devices, including energy-harvesting devices,[25, 38-42] semi-conductors,[43] light-emitting diodes,[44] molecular motors,[45] and hybrid conductive devices.[46] Among these, energy-harvesting devices are mostly studied.

Amyloid-templated metal-oxide semiconductor nanowires were successfully applied for creating photovoltaic devices.[25,38] For example, amyloid/titanium dioxide hybrid nanowires, together with organic semi-conductor polythiophene (P3HT), could be coated on poly 3,4-ethylenedioxy thiophene-poly styrene sulfonate (PEDOT-PSS) to form photovoltaic devices with superior performance.[25]

Organic semiconductor molecules could also be coupled with amyloids for building photoelectric devices. A rationally designed self-assembled amphiphilc peptide could cooperate with zinc protoporphyrin IX to produce a photovoltaic material.[39] A similar device integrating amyloid fibers/(5,15-diphenylporphyrin) Zinc could produce independent hole/electron pairs, facilitated by the introduction of TiO_2 into this system through biomimetic mineralization.[40]

In other cases, amyloid-like peptides or proteins conjugated with appropriate organic molecules can serve as active components for energy-harvesting devices.[41, 42] Channon et al. have rationally designed a fibrillar light-harvesting system by attaching functional organic molecules to an amyloidgenic peptide from transthyretin protein ($TTR_{105-115}$).[41] Kim et al. developed a light-harvesting system mimicking natural photosynthesis based on the diphenylalanine (FF), into which photosynthetic units including tetra (p-hydroxypehnyl) prohyyrin and platinum nanoparticles were purposely integrated.[42] This system could effectively regenerate the nicotinamide adenine dinucleotide dehydrogenase (NADH) under visible light.

Outlook

Several key engineering breakthroughs are needed to advance the use of amyloids for applications in bionanoelectronics. For example,

amyloid-enabled patterning technique has so far been limited to nanoparticles, organization and multi-scale patterning of nanowires, nanotubes and 2D nanomaterials has not yet been achieved. The coupling of bottom-up assembly with top-down patterning techniques may provide promising solutions. For example, chitin nanofibers assembly, when coupled with soft lithography, could produce multiscale patterning of nanofibers across several length scales, while actin self-assembly merged with top-down lithography has led to 3-dimensional nanoelectronic structures.[5, 7] In addition, various phage-based self-templating supramolecular structures have also been demonstrated when carefully manipulating their assembling processes at the air-liquid-solid interface.[4]

Furthermore, techniques for assembly of amyloid-based complex architectures that can match DNA-origami techniques[47] remain a challenge, despite their advantages in terms of mechanical and thermal stability. The coupling of amyloids with DNA-origami techniques may therefore present new opportunities.[48] Because amyloids can be engineered to bind to a wide range of atomically precise structures including DNAs, the development of self-assembled molecular composite nanosystems for constructing nanoelectronics with complex architectures may be within reach.

Techniques for multi-scale device fabrication and integration are also needed to put amyloid-enabled devices for practical applications. The ability of amyloids to form bulky films with hierarchical structures,[49] may lead to large-scale manufacturing of functional devices for real-life applications. This appears particularly important for achieving practical applications for amyloid-enabled energy-harvesting devices. In addition, more advanced electronic device architectures based on amyloids would also be expected in the future. For example, biosensors based upon nanowire field-effect transistors have been quite popular for sensing various molecules or biological objects,[50] but comparable amyloid-based devices have not yet appeared. In addition to the two overarching goals described here, another essential objective for bionanoelectronics is to deal with the interface between biology and nanoelectronic devices.[2, 51] It is likely that amyloid-enabled devices will be used for bio-interfacing applications in the future. Extra care will be required, however, because it is known that amyloids have been associated with several human diseases.[52]

Finally, an important area for bionanoelectronics is bioinspired nanoelectronics.[1, 5] Electronic signaling in biology often relies on molecular or celluar machines that harness proton or ionic currents.[53] Such natural systems have inspired and will continue to inspire new biomimetic functional electronic devices.[54] We envision that implementing this bioinspired-electronics strategy in amyloids will produce a new suite of devices with enhanced performance and novel functionalities, ultimately finding use in real-world energy, medical and environmental applications.

Acknowledgments

We sincerely apologize for any omissions of relevant work owing to space limitations. C.Z. acknowledges start-up funding support from ShanghaiTech University.

Notes and references

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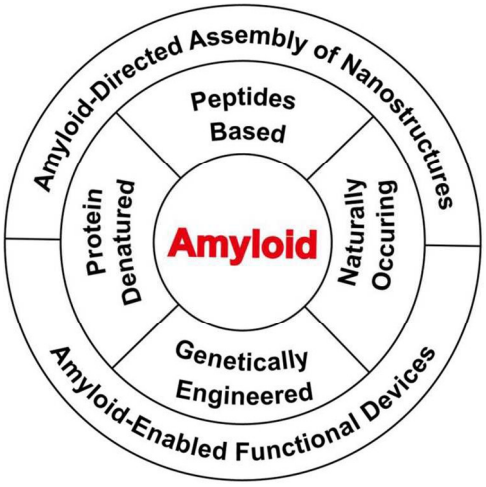

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